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An Experimental Study of the Size of the Pupil and Lens during Wolffian Lens Regeneration

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Three groups of adult *Triturus pyrrhogaster* were subjected to unilateral lentectomy (Group A), unilateral lentectomy and amputation of the tail (Group B) and bilateral lentectomy (Group C). These living animals were kept in water at 28°–30°C and the size of the pupil and the regenerated lens were studied from the 12th to 40th post-operative day using binocular microscopes. The results showed that in all stages, except the early stage, the area of the pupil and the projected area of regeneration were highly correlated, and therefore, the growth in size of the regenerated lens was greatly affected by the size of the pupil. However, the above-described experimental procedures had hardly any effect upon the size of the regenerated lens.

During the process of so-called Wolffian lens regeneration, a marked difference in size is frequently found among regenerates in the course of development even when animals with approximately the same size eye cup are used identical experimental conditions. STONE[6] (1954) has demonstrated that injury to the dorsal edge of the iris sustained at time of lens extraction is one of the important causative factors. However, the fact that even in experiments in which injury to the dorsal edge of the iris was avoided there is often a marked difference in size, suggests that this is not the sole cause and it is suspected that there are other causes.

As a mean of clarifying the cause, I have measured the size of the pupil and the lens during the course of regeneration and have obtained interesting results.

MATERIALS AND METHOD

The material consisted of adult female tritons *Trituruses pyrrhogaster* (Boie) captured in the suburbs of Nagasaki City and kept in a water tank for over half a year. Five series of experiments described below were conducted (table 1).

Each individual test animal was labelled with a number. In making the incision of the cornea, a transverse incision was made slightly below the center of the cornea so that the dorsal edge of the iris would not be caught in the incision wound of the cornea. All cases with even the slightest injury to the dorsal edge of the iris were omitted from the test series. The animals were classified into three test groups: cases in which only the lens of the right eye was removed (group A); cases in which the right lens was removed as well as amputation of the tail 13 mm from the tip (group B); and cases in which the left lens was removed as well as the right lens (group C). The animals were all kept in an incubator maintained at a water temperature of about 28 degrees C which is the temperature at which the rate of regeneration is most rapid (NAKAMURA[4], 1936). Observations using a stereoscopic microscope

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(eye piece lens × 8, objective lens × 3 or × 6) were done at a certain hour of the day on each animal every other day from the 12 th to 30 th day after operation and again on the 40 th day. With the use of a micrometer the area of the pupil and the projected area of the regenerated lens onto the pupil were measured directly on the living animal and the correlation between the two values was obtained. The pupil of a triton usually is a nearly round elliptical shape and therefore its area may be expressed as $\pi/4$ power of the product of the maximum diameter of the pupil and the maximum perpendicular diameter. The regenerated lens was assumed to be a sphere and projected area is expressed as $\pi/4$ power of the square of the transverse diameter. These measurements were carried out under certain definite illuminating conditions (Olympus 6V., 5A.). Until the 40 th day after operation, the pupil due to artificial light was not noted. In comparing the meaeurements for eaeh group, determination of whether there is a significant difference was accomplished by the application of the following equation:

TABLE 1.

Material

	Date of experiments (Water temperature)	Experimental animals				
		Test groups		Group A (Unilateral lentectomy)	Group B (Unilateral lentectomy and tail amputation)	Group C (Bilateral lentectomy)
I	29/V−8/VII (28°C)	No. of animals		9	10	5
		Length in Cm. (Crawn-Rump)	Minimum-Maximum	5.2−6.3	5.3−6.5	5.8−6.4
			Mean	5.8	6.1	6.2
II	21/VI−31/VII (28−29°C)	No. of animals		9	15	7
		Length in Cm. (Crawn-Rump)	Minimum-Maximum	4.5−6	5.3−6.2	4.3−5.7
			Mean	5.7	5.8	5.3
III	11/VII−6/VIII (28−30°C)	No. of animals		10	10	5
		Length in Cm. (Crawn-Rump)	Minimum-Maximum	5.4−6.2	5.5−6.1	5.2−5.9
			Mean	5.7	5.8	5.5
IV	29/VII−7/IX (28−30°C)	No. of animals		5	5	5
		Length in Cm. (Crawn-Rump)	Minimum-Maximum	5.2−6	4.8−6.3	5.1−5.9
			Mean	5.5	5.9	5.5
V	26/VIII−5/X (28−30°C)	No. of animals		9	8	4
		Length in Cm. (Crawn-Rump)	Minimum-Maximum	5.1−6	5.2−6.3	5.1−6
			Mean	5.7	5.6	5.5
	Totals of animals			42	48	26
	Mean	Length in Cm. (Crawn-Rump)	Minimum-Maximum	4.5−6.3	4.8−6.5	4.3−6.4
			Mean	5.7	5.5	5.6

$$\frac{M_1 - M_2}{\sqrt{m_1^2 + m_2^2}} > 2$$

where M is the mean and m is the sampling error. With regard to the correlation coefficient, a test of significance was made by the t-distribution table with the application of the equation :

$$t = \frac{r}{\sqrt{1 - r^2}} \sqrt{N - 2}$$

where r is the correlation coefficient and N is the number of cases.

RESULTS

The total number of animals in this experiment were 42 (42 eyes) in group A; 48 (48 eyes) in group B; and 26 (52 eyes) in group C (table 1), but among these animals there were some in which observation of the pupil was not possible due to opacity of the cornea or death during the course of the experiment. Therefore, the actual number of animals used for observation and measurement were 22–33 (22–23 eyes) in group A; 36–42 (36–42 eyes) in group B; and 13–14 (26–34 eyes) in group C. Thus total number of observations from the 12 th day to the 40 th day amounted to 325 eyes in group A, 433 eyes in group B and 342 eyes in group C (table 2 and 3).

1. Shape of pupil

The shape of the iris in each group until the 16 th day was normal, round or elliptical in some cases, but in many the shape was abnormal, being triangular, polygonal or irregular. From about the 18 th day, there was a gradual increase in the number of round or elliptical cases and on the 40 th day the great majority had morphologically recovered.

TABLE 2.

Area of the Pupils after Lentectomy in *Triturus pyrrhogaster*

Days after lens removal	Area of pupils in μ^2											
	Group A				Group B				Group C			
	No. of specimens	Mean	Standard deviation	Sampling error	No. of specimens	Mean	Standard deviation	Sampling error	No. of specimens	Mean	Standard deviation	Sampling error
12–day	28	230151	87244	16499	37	222606	101308	16654	26	238884	99404	19494
14–dry	29	227834	78150	14511	36	214796	99577	16596	28	210619	93600	17688
16–day	30	231938	71967	13138	38	217756	97096	15751	27	211713	97300	18724
18–day	31	236346	70087	12587	38	214885	93376	15147	34	190341	85213	14613
20–day	30	245206	82526	15067	41	217673	93636	14623	34	216705	76769	13165
22–day	31	261032	79683	14311	41	223887	86711	13541	31	231329	65791	11816
24–day	33	272822	84404	14693	41	259132	96063	15001	32	253154	69778	12334
26–day	33	290671	85590	14898	42	290945	93373	14407	34	265195	67113	11509
28–day	27	308813	68788	13238	39	306968	85414	13676	34	275826	68811	11800
30–day	27	327506	81167	15626	39	326775	86549	13859	33	312864	73020	12710
40–day	26	395458	77520	15202	41	408481	10034	15622	29	405633	94160	17484

2. *Area of pupil*

As shown in table 2, the average area of the pupil in each group until the 16 th day is not related in any definite fashion to the number of elapsed days, but after the 18 th day an increase in the area of the pupil was seen in accordance with the number of days following the operation and this rise in the curve continued until the 40 th day. However, the standard deviation and sampling error remained at a certain level irrespective of the number of elapsed days in all groups and consequently a tendency for the standard deviation and sampling error to gradually decrease was presumed for the late stage in view of the rate of increase of the area of the pupil, but each value still is considerably high indicating comparatively severe individual variations in the area of the pupil.

Next, when the mean value of each stage for each group is plotted on a growth curve (figure 1), the curve for group A shows a gradual increase after the 14 th day and is the highest of all three groups until the 28 th day but becomes the lowest on the 40 th day. In contrast to this, the curve for group C is generally the lowest of the three groups until the 30 th day, but on the 40 th day it almost approximates the value for group B which is the highest at this stage. The curve tor group B initially is located mid way between A and C, but from the 28 th day it approaches the curve for group A, finally exceeds it and on the 40 th day it is the highest. However, in spite of the difference in the height of these curves, no significant difference at the 5 per cent level could be demonstrated between the mean values of each group in any stage.

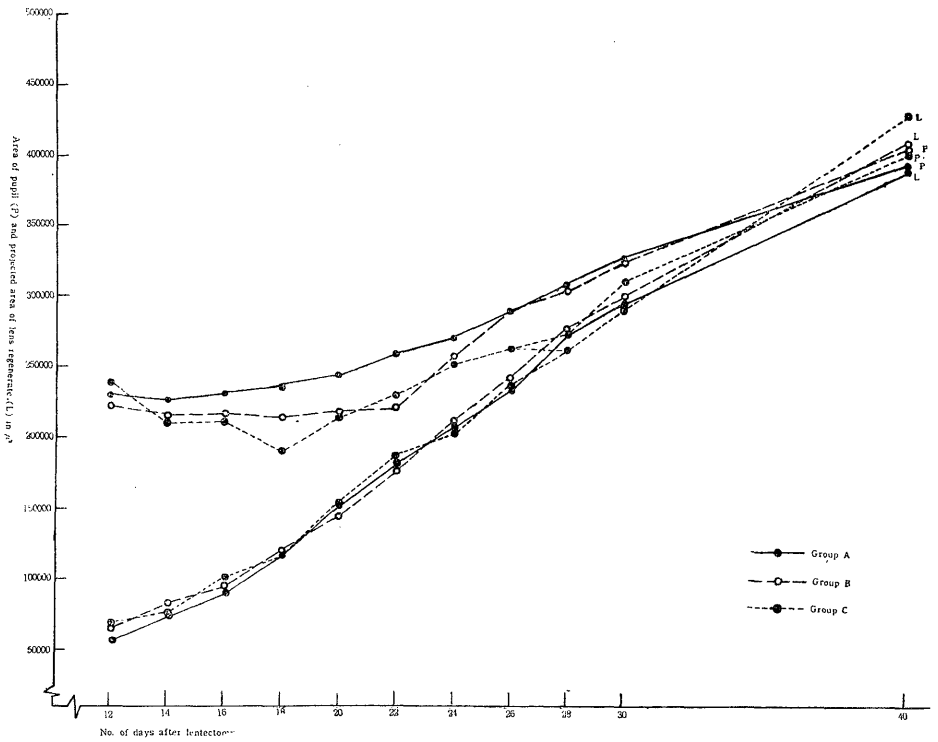

FIG. 1. Growth curves for the areas of the pupils and the projected areas of the lens regenerates after lentectomy in three experimental groups.

3. Shape of the regenerated lens

In the observation of the living animal, the regenerated lens until about the 12 th or 14 th day appeared in most cases to be a well defined, crescent or half-moon shaped, elevated, white spot on the mid-dorsal edge of the iris. After the 16 th day, due to the increasing size of the regenerated lens and indentation of the boundary between the lens and the edge of the pupil, it gradually appeared in the form of a perfect sphere suspended in the pupillary area. However, the upper part was hidden by the pigmented epithelial layer of the dorsal edge of the iris in some cases because of which it could not be seen from the outside as a perfect sphere until the last stage. The transverse diameter was used in the measurement of the projected area of the regenerated lens for this reason.

TABLE 3.

Projected Area of the Lens Regenerates after Lentectomy in *Triturus pyrrhogaster*

Days after lens removal	Projected area of lens regenerates in μ^2											
	Group A				Group B				Group C			
	No. of specimens	Mean	Standard deviation	Sampling error	No. of specimens	Mean	Standard deviation	Sampling error	No. of specimens	Mean	Standard deviation	Sampling error
12–day	28	58693	32467	6135	37	67289	35429	5824	26	68044	30895	6057
14–day	29	75187	28887	5364	36	83473	26100	4349	28	75874	33837	6394
16–day	30	90845	33694	6151	38	95808	40162	6482	27	102881	40156	7727
18–day	31	118177	41920	7528	38	119714	46172	7489	34	118157	44444	7621
20–day	30	152970	51508	8596	41	145313	56654	8847	34	153540	49856	8553
22–day	31	184048	53036	9525	41	177696	58777	9178	31	187342	48568	8722
24–day	33	209309	61188	10650	41	212259	57770	9021	32	205509	56701	10023
26–day	33	236026	67310	11716	42	243920	67383	10397	34	236331	62300	10683
28–day	27	275950	69998	13471	39	278291	65374	10467	34	264355	65362	11208
30–day	27	298569	78445	15096	39	308494	71910	11514	33	292428	55346	9634
40–day	26	393328	64854	12718	41	411569	91637	14310	29	433344	83585	15521

4. Projected area of the regenerated lens

The projected area of the regenerated lens increased with lapse of time following operation in all groups, but the standard deviation and sampling error increased along with the increase in the projected area of the regenerated lens which indicated the increasing individual variation (table 3).

When the average projected area of the regenerated lens in the respective stages for each group is shown on a growth curve (figure 1), the values for all three groups are almost the same. Except for the mild elevation in the curve for group C in the initial period, the curves generally overlap each other as they gradually rise. However, in the last stage there was considerable difference in height, being highest in group C followed by group B with group A being the lowest. Even so, there was no significant difference.

As to the relationship with the area of the pupillary area, the porjected area of the regenerated lens in the early stage was much smaller than the area of the pupil but subsequently due to the marked increase in size, the difference gradually decreased and the projected area of the regenerated lens approximated or exceeded the area of

the regenerated lens.

TABLE 4.

Statistical Analysis of Correlations between Area of the Pupils and Projected Area of the Lens Regenerates after Lentectomy in Each Stage by Means of Student's t-Distribution Table

Days after lens removal	Group A					Group B					Group C				
	No. of specimens	r	t	Limit of significance (5% level)	Significance	No. of specimens	r	t	Limit of significance (5% level)	Significance	No. of specimens	r	t	Limit of significance (5% level)	Significance
12–day	28	0.514	3.055	2.056	+	37	0.657	5.155	2.032	+	26	0.323	1.671	2.064	−
14–day	29	0.386	2.173	2.052	+	36	0.702	5.746	2.034	+	28	0.193	1.002	2.056	−
16–day	30	0.273	1.501	2.048	−	38	0.539	3.839	2.029	+	27	0.401	2.182	2.060	+
18–day	31	0.477	2.922	2.045	+	38	0.658	5.242	2.029	+	34	0.400	2.461	2.038	+
20–day	30	0.478	2.878	2.048	+	41	0.728	6.629	2.023	+	34	0.601	4.242	2.038	+
22–day	31	0.500	3.214	2.045	+	41	0.701	6.123	2.023	+	31	0.680	5.162	2.045	+
24–day	33	0.633	4.551	2.040	+	41	0.562	4.242	2.023	+	32	0.691	5.235	2.042	+
26–day	33	0.708	5.581	2.040	+	42	0.733	6.916	2.021	+	34	0.764	6.688	2.038	+
28–day	27	0.705	7.007	2.060	+	39	0.739	6.671	2.027	+	34	0.725	5.968	2.038	+
30–day	27	0.839	7.710	2.060	+	39	0.762	7.157	2.027	+	33	0.813	7.773	2.040	+
40–day	26	0.819	6.990	2.064	+	41	0.892	12.318	2.023	+	29	0.834	7.853	2.052	+

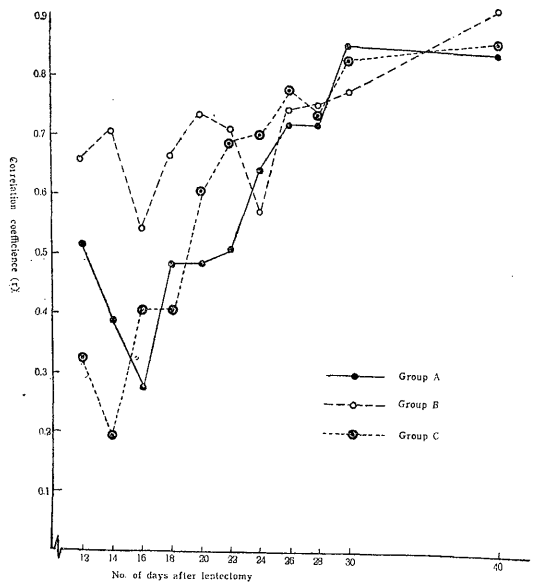

FIG. 2. Changes in the correlation values between area of the pupils and projected area of the lens regenerates during regeneration in three experimental groups.

the pupil between the 30th and 40th day in all three groups.

5. Correlation between area of pupil and projected area of the regenerated lens

The correlation coefficient between the area of the pupils and the projected area of the lenses in each stage was found to be significant in all groups except on the 16th day in group A and on the 12th and 14th day in group C (table 4). With regard to changes in the correlation coefficient with lapes of time (figure 2), the values were high throughout the entire course in group B but were the lowest on the 16th day in group A and on the 14th day in group C with the least correlation between the area of the pupil and the projected area of the lens in the preceding and following stages in both of these two groups. Except for a considerable fluctuation in group B, there subsequently was a rapid rise until

the 26 th day with a high degree of correlation in all three groups up to the last stage.

DISCUSSION

Detailed histological studies (FISCHEL[1], 1900; KOJIMA[3], 1939; etc.) have been done on the changes of the edge of the iris in the period prior to the commencement of regeneration following lens extraction in Urodela amphibia, but the only report on the size of the pupil following lens regeneration seems to be the statement that the pupil which rapidly decreases after operation recovers to almost normal between the 18 th to 21 st day in Anura amphibia (KITAZATO[2], 1940). Furthermore, there is no report on the correlation between the pupil and the lens.

In the present experiment, a total of 119 eyes of 92 animals kept at a water temperature of 28–30 degrees C were observed from the early stage of lens regeneration, the 12 th day, up to the 40 th day and during this period, measurements of the area of the pupil and the projected area of the the lens were taken on the living animals. Between the 12 th day to 16 th day no definite relation was found between the area of the pupil and the number of days elapsed from operation, but after the 18 th day an increased relationship was noted with the lapse of time. On the other hand, the projected area of the regenerated lens in the early stage was much smaller than the pupil, with the lapse of time there was a rapid increase in size which approximated or exceeded the size of the pupil between the 30 th to 40 th day. The fact that the increase of the area of the pupil continued throughout the last stage of regeneration indicates that the repair of the iris in this triton is slower than in Anura amphibia (KITAZATO[2], 1940) and requires a period of at least more than 40 days.

The correlation coefficient between the size of the pupil and the regenerated lens was obtained for each stage in each case. It was found to be significant in all stages except the early stage. In particular, there was a high correlation in the later stages. In view of this fact, it is assumed that when the recovery of the size of the pupil is delayed due to the mechanical oppression or injury to the pupillary margin of the iris sustained at time of lens extraction, the growth in size of the regenerated lens also is disturbed as a result of which there are differences in the size of the lens in accordance with the size of the pupil even among cases that had been operated on at the same time. That is, in addition to the delay in the growth of the regenerated lens due to the injury to the dorsal edge of the iris presented in past reports (STONE[6], 1954), the size of the pupil also should be taken into consideration as being an important factor which influences the growth in size of the lens. In this case, however, it is conceivable that the delay in the growth of the size of the lens due to the delay in the recovery of the pupil may be because the energy which should be used in lens regeneration is partly utilized for the recovery of the size of the pupil. However, it seems more appropriate to interpret this as a phenomenon of harmony of size of various parts of the body of living animals since even when the shape of the pupil is irregular the size of the regenerated lens generally is in proportion to the pupil just as a proportionotely small lens develops when the dimensions of the eye cup which had formed is small in Bombinator pachypus in which the brain anlage of the right side had been excised in the germinal stage (SPEMANN[5], 1912).

The great possibility of errors due to difficulty of measurement because of the

variability in the size and shape of the pupil id a great number of cases caused by the inflammation of the pupillary margin of iris resulting from the operative procedure until the early stage of regeneration perhaps may be responsible for the lack of a definite relationship between the area of the pupil and the number of days elapsed as well as the low correlation coefficient in this stage of regeneration in this experiment.

A further review was done to determine whether there is any difference in the size of the regenerated lens between cases in which only unilation had been done (group A), cases in which amputation of the tail had been done in addition to unilateral lens extraction (group B) and cases in which bilateral lens extraction (group C) had been done. It was found that the mean size of the pupil in the first half of regeneration was in the order of A>B>C with a difference in the rate of recovery of the pupil, but the mean projected area of the regenerated lens showed hardly any difference at all except on the 40th day when the order was C>B>A. However, no statistically significant differences were found between these values. Consequently, whatever procedure may be done, whether unilateral lens extraction, injury to some other part of the body requiring repair in addition to lens extraction, or bilateral lens extraction, there is hardly any effect upon the growth of the size of the itself even if there may be some effect upon the repair of the pupil.

CONCLUSION

Studies were done on three groups of adult *Triturns pyrrhogaster* (Boire); those on which unilateral lens extraction had been done, those on which amputation of the tail had been done in addition to unilateral lens extraction, and those on which bilateral lens extraction had been done. These animals had been kept at a water temperature of 28–30 degrees C and observations were made on the size of the pupil and regenerated lens using microscopes on the living animal. The following findings were obtained.

1) Light reflex of the pupil following lens extraction was absent until the 40th day.

2) Between the 12th to 16th day no definite relationship was noted between the area of the pupil and the number of days elapsed, but after the 18th day there was an increased relationship with lapse of time. Consequently, the time required until the pupil recovered to normal was felt to be more than 40 days.

3) From the 12th day the projected area of the regenerated lens increased with the lapse of time and approximated or exceeded the size of the pupil between the 30th and 40th day.

4) In all stages, except the early stage, the area of the pupil and the projected area of the regenerated lens were highly correlated particularly in the later half and it is assumed that the growth in the size of the regenerated lens is greatly affected by the size of the pupil.

5) The mean projected area of the regenerated lens for each test groups revealed no significant difference throughout the entire course. Therefore, it is felt that the previously described experimental procedures have hardly any effect upon the size of the regenerated lens.

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REFERENCES

1) FISCHEL, A. : *Anat. Hefte* 14 : 1–255. (1990).
2) KITAZATO, Y. : *Nagasaki Igakkai Z.* 18 : 38–49 (1940) (Japanese).
3) KOJIMA, T. : *Nagasaki Igakkai Z.* 17 : 2522–2545 (1939) (Japanese).
4) NAKAMURA, O. : *Doubutsu & Shokubutsu* 5 : 32–40 (1936) (Japanese).
5) SPEMANN, H. : *Zool. Jahrb.* 32 : 1–98 (1912).
6) STONE, L.S. : *Anat. Rec.* 120 : 599–624 (1954).